Supplemental Figure and Tables

Table S1. Influence of mucin on MIC. Liquid MIC results done in SCFM with 0.4% mucin and without mucin grown at 37°C overnight with an inoculum size of 2-7 x 10^5cells (n=3-5).

Antibiotic	MIC (μg/ml)	
	+ mucin	- mucin
Gentamicin	4	1
Tobramycin	2	2
Amikacin	16	4
Imipenem	0.325	0.625
Meropenem	0.125	0.125
Ceftazidime	31.25	31.25
Aztreonam	4	8
Piperacillin	4	4
Erythromycin	500	250
Clarithromycin	2000	2000
Polymyxin B	16	16
Colistin	16	2
Norfloxacin	16	16
Ciprofloxacin	1	0.5
Trimethoprim	128	128
Tetracycline	64	256
Chloramphenicol	32	16

Table S2. Resistome mutant susceptibility under surfing conditions - raw data. Average zone of inhibition measurements for resistome mutants tested against five selected antibiotics. Mutants of up-regulated resistome genes were tested against 10 μg/disk of antibiotic and down-regulated against 100 μg/disk. Statistical significance relative to wild-type was determined using two-way ANOVA. (n=3 resistome mutants; n=6 wild-type) * $p<0.5$, ** $p<0.01$, *** $p< 10^{-3}$, **** $p<10^{-4}$. Standard deviations range from 0 to 2.5mm.

Mutant	Zone of Inhibition (mm)				
	Imipenem	**Tetracycline**	**Polymyxin B**	**Tobramycin**	**Norfloxacin**
10 μg/disk antibiotic concentration					
Wild-type	5.7	5.0	5.6	3.3	1.0
ΔrecG	7.3	8.7*	9.7**	12.5****	7.3****
ΔddaH	9.0*	0***	5.3	3.0	2.3
ΔPA5130	10**	0***	0****	0*	6.5****
cycH	4.5	4.3	11****	9.7****	0
100 μg/disk antibiotic concentration					
Wild-type	12.3	6.7	8	12	14.7
ΔarmR	0****	0****	1****	6.3****	0****
ΔPA3576	12.0	3.0*	6.0	8.3*	10.7*
ΔPA1428	12.7	7.7	8.0	7.0***	0.0****
ΔPA2047	12.3	7.0	5.7	7.3**	9.7***
ΔPA1553	9.0	7.3	7.7	8.0*	10.5*
*ΔatpB**	9.7	4.0	8.0	8.3*	9.7***
ΔPA4292	7.7**	5.3	17****	5****	10**
ΔclpS	8.3*	6.3	15****	6.7***	8.3****
ΔnuoB	10.7	0****	18****	6.3****	10.3**
ΔPA3721	10	2**	14.5****	0****	10**
ΔPA4429	11	8.7	20****	0****	7.7****
ΔetfA	12.3	9	15****	7.3**	10**
ΔnuoG	9.7	6.5	6	0****	7.3****
ΔPA4781	12.3	10	10.3	6.7***	9****
ΔserA	14.7	8.7	12.7**	7.3**	11*
ΔccmF	14	8.7	13.3***	7.3**	8.7****
ΔPA3667	15.7	0.0****	7.7	10.0	12.0
ΔPA1513	10.7	0.0****	7.3	10.7	14.0
ΔpchF	9.7	5.0	7.3	1.1****	13.3
Δrph	13.0	9.0	6.0	5.0****	15.0
ΔPA2566	11.0	4.0	7.0	9.3	9.7***
ΔgidA	9.7	7.3	9.0	10.5	10.0**
ΔmutS	16.0	9.3	4.3*	6.3****	11.5
ΔthiG	6.3****	6.7	7.0	8.7	10.3**
ΔnuoF	11.0	5.3	6.7	7.0***	14.7
ΔpckA	9.7	4.8	7.0	6.3****	12.0
ΔPA2571	12.7	6.7	7.0	7.3**	11.7

ΔPA4766	13.7	6.0	6.5	7.0***	13.7
ΔPA1348	16.7**	5.5	8.3	10.7	10.7*
ΔbraB	11.0	6.7	5.7	10.0	10.7*
ΔhtpX	12.7	5.7	8.3	11.0	9.5**
ΔspeA	11.3	4.5	5.3	10.3	12.0
ΔadhA	12.3	7.0	6.7	10.3	13.3

Table S3. RT-qPCR results confirmed the dysregulation of resistome genes shown in RNA-Seq. The relative fold-change of expression of select resistome genes under surfing conditions (SCFM + 0.4% mucin) relative to swimming (SCFM 0.3% agar) from both the RNA-Seq experiment and RT-qPCR of cells collected from the centre and edge of a surfing colony relative to swimming cells. (FC cut-off of RNA-Seq is ± 1.5).

	Gene Expression (FC)			
	RT-qPCR		**RNASeq**	
Gene	**Centre**	**Edge**	**Centre**	**Edge**
recG	16.4	8.1	1.9	2.1
PA5130	2.2	1.5	NC	2.4
ddaH	2.8	3.2	4.9	2.4
PA1428	-2.2	-3.3	-3.4	NC
PA2047	3.1	-1.4	NC	-2.1
thiG	-2.5	1.8	-2.9	NC
PA3667	-1.2	-3.4	-1.7	-2.5
PA3576	2.3	-4.4	NC	-2.9
atpB	-1.5	1.1	-2.1	NC
PA4292	-2.3	-2.4	-6.7	NC
nuoB	-1.0	-1.2	-2.8	NC
PA3721	-2.0	2.1	-5.3	-2.7
clpS	5.0	-3.8	NC	-2.3
armR	-4.4	2.9	-3.2	-5.1
cycH	5.7	1.4	NC	2.2

Table S4. Primers used in this study. Primers used for PCR amplification are denoted as F (forward) and R (reverse). All primers used for quantitative PCR (qPCR) are denoted as qF (forward) and qR (reverse).

Primer Name	Sequence (5'-3')
armR_F	AGCGTGGCGCCGGAC
armR_R	CTGCGCGGATTCTGATAGCTCA
armR_qF	CTGAACACTCCGCGCAACAA
armR_qR	GTGTAATCCCGCCGACCGAC
atpB_F	GCTTCGGGTTACATCCAGCA
atpB_R	CGGCGATAGCAGTCAGTCC
atpB_qF	TGAAGATGAAGGCCTGCAGG
atpB_qR	TCGGTAGCGGAATGTTCCTG
clpS_F	TTGCAGTTAGTCAAGGCGCT
clpS_R	AAGGCGAGATTGAGGGTGAC
clpS_qF	TCAATCAGGACCATCCCGAG
clpS_qR	AACGGTGGCGGCTGTAATAC
cycH_qF	TAGACAACGGTTTGCCGCGT
cycH_qR	CAGCTCAAGCTCAGCGGATT
ddaH_F	CGATTTATCAAGGATTTCCAAGGCA
ddaH_R	CCTCCGAAACTGCATCCG
ddaH_qF	ATCATCGAGGAGACCGTGCA
ddaH_qR	TGGTCGCCGACCATCATGAT
nuoB_qF	CGCGTTTCAGGTCTTTCTGG
nuoB_qR	TGCTGCTGCAGGAATCCATC
PA1428_F	GCGACTGAAGCGTACATGCC
PA1428_R	ACTTCACCCGTTTCTTCAAGC
PA1428_qF	TTCTGCTTCTGCAACTCGGC
PA1428_qR	AAGATCCGCCGCTACATTCC
PA2047_F	CTTGCCCATTCGTGCCGAT
PA2047_R	TGTATTGCAAGCGCCCCC
PA2047_qF	AAGGTGCCGATTTCCACGTC
PA2047_qR	AACGCTACCTGGTGATGTCG
PA3576_F	CCGTCCCGACTTGTCCCG
PA3576_R	CGAAACGCCACCTTCGTGC
PA3576_qF	TTGCAGGTTGCCGACCAGTT
PA3576_qR	ATCGGCTATGCGGTGCTGAA
PA3667_F	ACCCTTTCCCCTGTAACCGT
PA3667_R	TGTTCCCAACTGGCGGTG
PA3667_qF	GAAGCAGGTTGGCATGGTGT
PA3667_qR	ACAGAGGCGATCAACCTGGT
PA3721_F	GCTCTCAGCAAACCGCCT
PA3721_R	CGAGATCCACCTCACCGATCT

PA3721_qF	TCCAGGTAGCAGGCGATGAT
PA3721_qR	AAGGCCGACCTGTTCCTCAA
PA4292_qF	GTTCGATCTTTTCAGCGGGC
PA4292_qR	CGTGGAAGCCGTTGATGAAC
PA5130_qF	ATGGCGTCGACCAGGATGAT
PA5130_qR	ACATCGTCGACGCGCTGAAC
recG_F	GTCCACGCGCCATCGAA
recG_R	CAAGACTGAGACCTACGCCG
recG_qF	CTGGAGATCTACCATCCGGA
recG_qR	TGGGTGAGTCCTTCGGTAGT
thiG_F	GATCGTCCACGCCATCGG
thiG_R	CGCCCCAGGTAGTCGGTAT
thiG_qF	TGAGCCAAGCATCCAGTACC
thiG_qR	GTCTCGTCGAGGTCCTTGTA

Table S5. Concentrations of the antibiotics in the disk diffusion assay including their solvents. Ten µL of antibiotics was added per disk and dried prior to application onto agar surfaces. MeOH – methanol. DMSO – dimethyl sulfoxide.

Antibiotic	Concentration (µg/disk)	Solvent
Gentamicin	10	Water
Tobramycin	10	Water
Amikacin	5	Water
Imipenem	10	Water
Meropenem	5	Water
Ceftazidime	5	Water
Aztreonam	30	Water
Erythromycin	1000	MeOH
Clarithromycin	500	Water
Polymyxin B	10	Water
Colistin	10	Water
Norfloxacin	5	Water
Ciprofloxacin	10	Water
Trimethoprim	1000	DMSO
Tetracycline	10	MeOH
Chloramphenicol	5	Water

Fig S1. Disk diffusion assay plate set-up. Mid-log phase (OD_{600}=0.4-0.5) cultures are inoculated at 1uL around the antibiotic disk at equal distances. Four-point inoculation was used for swim and surf antibiotic disk assays. Disk diffusion control assays were done using a bacterial lawn spread with 50uL of mid-log phase culture and dried antibiotic disks were applied to the centre of each plate.